Australian Government
Department of Health
Australian Industrial Chemicals Introduction Scheme

2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-, compounds

Evaluation statement

30 June 2022

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AICIS evaluation statement

Subject of the evaluation

2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-, compounds

Chemicals in this evaluation

Name	CAS registry number
2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-	642-59-1
2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-, trisodium salt	915-67-3
2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-, aluminum complex	12227-62-2

Reason for the evaluation

The Evaluation Selection Analysis indicated a potential risk to human health.

Parameters of evaluation

These chemicals are listed on the Australian Inventory of Industrial Chemicals (the Inventory). This evaluation is a human health risk assessment for all identified industrial uses of these chemicals in Australia. These chemicals have been assessed as a group based on similarities in structure and end use patterns.

Summary of evaluation

Summary of introduction, use and end use

These chemicals are listed on the Personal Care Products Council ingredient database with functions as colourant. The trisodium salt of amaranth and lake pigment (CAS Nos. 915-67-3; 12227-62-2) have been identified in personal care products in Australia and internationally. Reported products include:

- soaps
- bath bombs
- hand and body wash
- facial cleansers and eye cream
- make up (including in lipstick).

Available data indicate use at low concentrations with a maximum concentration of 3% reported in lipstick products. The trisodium salt has reported domestic (including in washing and cleaning products) and commercial (including in putties and fillers) uses, although use in

these products does not appear to be widespread. These chemicals also have reported commercial uses including in the manufacture of textiles and site limited uses in the manufacture of chemical substances.

The trisodium salt and lake pigment may be used in permanent make up (PMU) inks and in tattoo inks. However, none of these chemicals in this group were identified as present in tattoo or PMU inks available in Australia.

There are reported non-industrial uses for the trisodium salt including in pesticides and therapeutic goods, and as food additives.

Human health

Summary of health hazards

The critical health effects for risk characterisation include potential eye irritation effects.

Based on an in vitro study on the trisodium salt of amaranth, chemicals in this group may cause eye irritation. This chemical (neat) applied to reconstructed human cornea like epithelium (RhCE) resulted in a reduced mean tissue viability (17.8%). Further information would be required for classification purposes, and local effects such as eye irritation cannot necessarily be read across.

Chemicals in this evaluation contain an azo bond with sulfonic acid moieties each side of the azo bond. Aromatic amines produced following reduction of the azo bond would be sulfonated and; therefore, be water soluble, have low bioavailability through the skin and be readily excreted. The major metabolite is naphthionic acid (1-amino-4-naphthalenesulfonic acid, (CAS No. 84-86-6). The other metabolite from reductive cleavage of the azo bind is expected to be 1-amino-2-hydroxy-3,6-naphthalenedisulfonic acid (CAS No. 135-51-3).

No data are available for the other chemicals in the group. The toxicological studies using the trisodium salt was used to read across for the other chemicals in the group for systemic toxicity.

Based on the available data for the trisodium salt of amaranth, chemicals in the group are expected to have low acute oral toxicity (median lethal dose (LD50) >2000 mg/kg body weight (bw) in mice). Based on the low acute oral toxicity and expected limited bioavailability through the skin these chemicals are expected to have low acute dermal toxicity.

Chemicals in the group are not expected to be skin irritants. In an in vivo study, rabbits treated with the trisodium salt at doses of 0.1% or 1% did not show skin irritation. In an in vitro skin irritation study the trisodium salt was determined to not be irritating to the skin. There is no evidence of skin sensitisation in a single non-guideline study. The in silico data indicates that these chemicals may have weak sensitisation potential.

A large number of non-guideline studies are available for the trisodium salt in several species investigating carcinogenicity, repeated dose toxicity, and reproduction and developmental toxicity. Based on a weight of evidence these chemicals are not expected to cause serious systemic effects following repeated exposure, carcinogenic effects or specific adverse effects on fertility and development. Based on the weight of evidence from in vitro and in vivo studies for the trisodium salt and in silico data for the free acid and its metabolites, these chemicals are not expected to have genotoxic potential.

Hazard classifications relevant for worker health and safety

These chemicals do not satisfy the criteria for classification according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UNECE 2017) for hazard classes relevant for work health and safety. This evaluation does not consider classification of physical hazards and environmental hazards.

Summary of health risk

Public

Based on the available use information, the public may be exposed to these chemicals in this evaluation by:

- direct application of cosmetic products to the skin, lips or hair
- tattoo or PMU ink application within the skin
- incidental skin and eye exposure to these chemicals during use of domestic products.

At the concentrations likely for these chemicals in this group, local effects (eye irritation) are not expected. Therefore, there are no identified risks to the public that require management.

Workers

During product formulation, ocular exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to these chemicals at lower concentrations could also occur while using formulated products containing these chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the potential local health effects (eye irritation), these chemicals could pose a risk to workers. Control measures to minimise ocular exposure are needed to manage the risk to workers (see **Proposed means for managing risk** section).

Proposed means for managing risk

Workers

Information relating to safe introduction and use

The information in this statement should be used by a person conducting a business or undertaking at a workplace (such as an employer) to determine the appropriate controls under the relevant jurisdiction Work Health and Safety laws.

Control measures that could be implemented to manage the risk arising from exposure to these chemicals include, but are not limited to:

- minimising manual processes and work tasks through automating processes
- adopting work procedures that minimise splashes and spills
- cleaning equipment and work areas regularly
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with these chemicals.

Measures required to eliminate or manage risk arising from storing, handling and using these hazardous chemicals depend on the physical form and how these chemicals are used.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk.

Model codes of practice, available from the Safe Work Australia website, provide information on how to manage the risks of hazardous chemicals in the workplace, prepare an SDS and label containers of hazardous chemicals. Your Work Health and Safety regulator should be contacted for information on Work Health and Safety laws and relevant Codes of Practice in your jurisdiction.

Conclusions

The conclusions of this evaluation are based on the information described in this Evaluation Statement.

Considering the proposed means of managing risks, the Executive Director is satisfied that the identified human health risks can be managed within existing risk management frameworks. This is provided that all requirements are met under environmental, workplace health and safety and poisons legislation as adopted by the relevant state or territory and the proposed means of managing the risks identified during this evaluation are implemented.

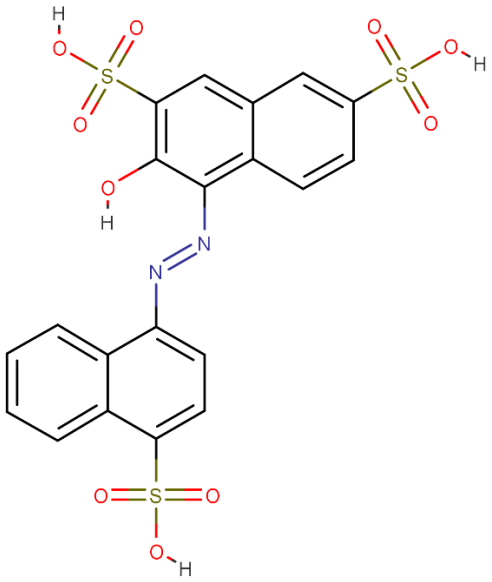
Note: Obligations to report additional information about hazards under *Section 100* of the *Industrial Chemicals Act 2019* apply.

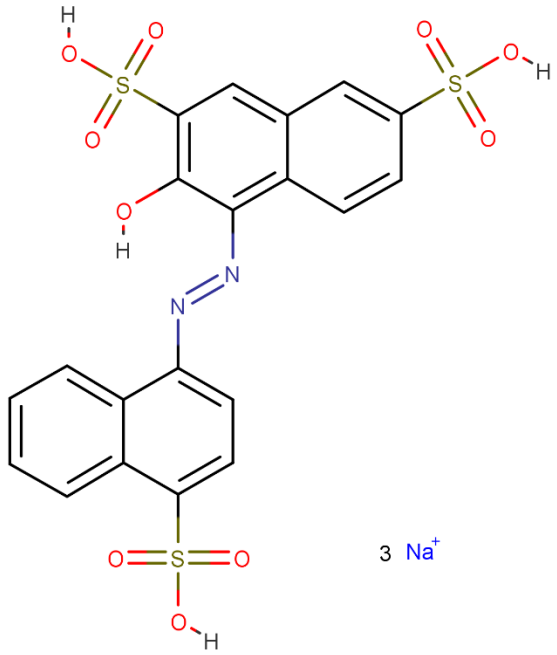
Supporting information

Grouping rationale

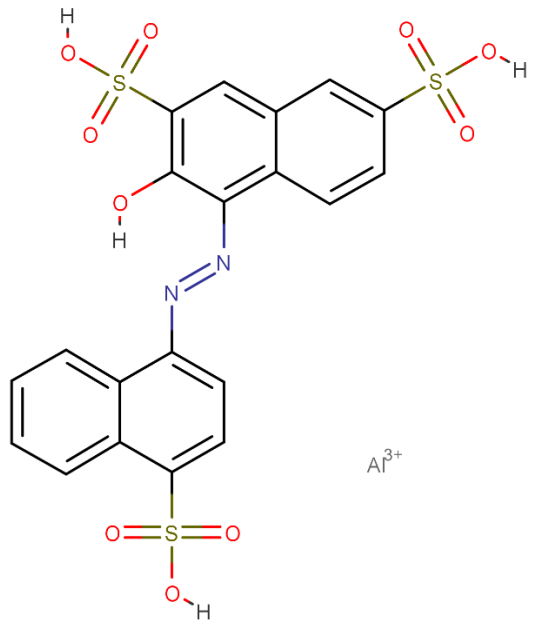
Chemicals in this group share a common moiety: 2,7-naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-. These chemicals have sulfonic acid substituents (SO_3-) at 3 positions. Two chemicals are azo dyes (CAS No. 642-59-1 and 915-67-3) and one (CAS No. 12227-62-2) is a lake pigment. These chemicals are all considered to be able to undergo reductive enzymatic cleavage by the skin or gut microflora or the azoreductase enzymes within the various organs (see **Toxicokinetics** section), although the extent of reduction may vary. The majority of data are available for the trisodium salt. Given the structural similarity it is considered appropriate to read across this data to other chemicals in the group for systemic toxicity.

Chemical identity

Chemical name	2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-
CAS No.	642-59-1
Synonyms	C.I. Acid Red 27, free acid Amaranth, free acid 2-naphthol-3,6-disulfonic acid, 1-(4-sulfo-1-naphthylazo)- (6CI) 3-hydroxy-4-[2-(4-sulfo-1-naphthalenyl)diazenyl]-2,7-naphthalenedisulfonic acid
Structural formula	
Molecular formula	C20H14N2O10S3
Molecular weight (g/mol)	538.53
SMILES	O=S(=O)(O)C=1C=CC=2C(C1)=CC(=C(O)C2N=NC3=CC=C(C=4C=CC=CC34)S(=O)(=O)O)S(=O)(=O)O

Chemical name	2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-, trisodium salt
CAS No.	915-67-3
Synonyms	C.I. 16185 C.I. Acid Red 27, trisodium salt Amaranth
Structural formula	
Molecular formula	C20H14N2O10S3.3Na
Molecular weight (g/mol)	607.5
SMILES	[Na].O=S(=O)(O)C=1C=CC=2C(C1)=CC(=C(O)C2N=NC3=CC=C(C=4C=CC=CC34)S(=O)(=O)O)S(=O)(=O)O

Chemical name	2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-, aluminum complex
CAS No.	12227-62-2
Synonyms	C.I. 16185:1 C.I. Pigment Red 193 Acid Red 27 Aluminium lake C.I. Acid Red 27, aluminium complexes C.I. Food Red 9, aluminium lake Certolake amaranth

Structural formula	
Molecular formula	C20H14N2O10S3.Al
Molecular weight (g/mol)	565.51

Relevant physical and chemical properties

Based on data for the trisodium salt, the azo dyes are solids at room temperature. They have high water solubility (50000 mg/L, log Kow = -5.1) and low volatility (estimated vapour pressure 5.82 × 10^{-25} mmHg) (REACHa). The lake pigment (CAS No. 12227-62-2) is also a solid at room temperature with low volatility (estimated vapour pressure 4.51 × 10^{-23} mmHg). The lake pigment has low water solubility (0.002 mg/L, log Kow = 4.99) (REACHb).

Introduction and use

Australia

The trisodium salt and lake pigment are ingredients in personal care products (including soaps, bath bombs, hand and body wash) as reported in publicly available information. The trisodium salt is reported in publicly available Australian safety data sheets (SDS) for dye products.

International

Chemicals in this group are listed on the Personal Care Products Council database as colourants. The function of the lake pigment in the CosIng database is hair dyeing. The dyes (CAS No. 915-67-3; 642-59-1) are listed as colourants (CosIng).

The trisodium salt and lake pigment have the following reported cosmetic uses including in (CosIng; DeLima Associates; EWG; Government of Canada 2016; REACHa; REACHb):

- perfumes and fragrances

- eye, lip and face make up (lipstick ≤3%)
- bath products, personal hygiene products and soaps
- cleansers
- creams and moisturisers (at concentrations of ≤0.1%)
- face paint
- hair dyes, hair grooming and hair removal products
- massage oils
- nail care products.

Where available the position of the chemical on the label information indicates that the chemical is used in low concentrations.

The trisodium salt may be used in PMU inks (Government of Canada 2016). The lake pigment may be used in tattoo inks as reported in publicly available information. In the NICNAS reports (2017; 2018), tattoo and PMU inks likely to be used in Australia were analysed by identifying ink brands from Australian customs importation data and online sellers, conducting a survey of a group of Australian tattoo artists and PMU professionals, and conducting chemical analysis of tattoo and PMU inks. However, no chemicals in this group were identified as present in the sample of tattoo or PMU inks available in Australia (NICNAS 2017; NICNAS 2018).

The trisodium salt has the following reported domestic uses including in (REACHa):

- air fresheners
- biocides (e.g. disinfectants)
- coating products
- modelling clay
- finger paints
- inks and toners
- polishes and waxes
- automotive care products
- washing and cleaning products.

There were no domestic uses reported in North American consumer product information database (DeLima Associates).

The trisodium salt has the following reported commercial uses including (REACHa; SPIN):

- in fillers, putties, plasters
- as surface treatments
- in wholesale and retail trade and repair of motor vehicles and motorcycles
- in building and landscape materials
- dyeing of textiles, leather and fur.

The trisodium salt and lake pigment have the site limited uses in the manufacture of chemical substances (REACHa; REACHb):

The trisodium salt has the following reported non-industrial uses including in pesticides and preservatives and therapeutic goods, and as a food additive.

Existing Australian regulatory controls

AICIS

No specific controls are currently available for these chemicals.

Public

No specific controls are currently available for industrial use of these chemicals.

The trisodium salt is permitted for use as a food additive under Schedule 15 of the Food Standards Code (FSANZ).

Workers

Chemicals in this group are not listed on the HCIS and no specific exposure standards are available (SWA).

International regulatory status

Exposure standards

No specific exposure standards were identified for chemicals in this group.

European Union

The dyes are listed in Regulation (European Commission (EC)) 1223/2009 on cosmetic products, Annex IV – List of colorants allowed in cosmetic products (EC 2021a).

The trisodium salt, when used as a substance in hair dye products, is listed in Regulation (EC) 1223/2009 on cosmetic products, Annex II – List of substances prohibited in cosmetic products (EC 2021b).

According to EU Regulation 2020/2081, substances listed in Annex II of Regulation 1223/2009 that is present in the mixture at a concentration ≥0.00005% (by weight) shall not be placed on the market in mixtures used for tattooing purposes, and mixtures containing any such substances shall not be used for tattooing purposes. Therefore, trisodium salt at a concentration ≥0.00005% (by weight) is not permitted for use in tattoo inks or PMU (EC 2020). The Committee for Risk Assessment (RAC) was of the opinion that the exemption of 21 colourants that are listed in Annex II of Regulation 1223/2009 (including the trisodium salt) cannot be based on their non-hazardous data, primarily due to the lack of adequate information on their hazard properties and risk for human health (ECHA 2018).

New Zealand

The trisodium salt is listed in the New Zealand Cosmetic Products Group Standard, Schedule 6 – Colouring agents cosmetic products may contain with restrictions (New Zealand EPA 2019).

The trisodium salt when used as a substance in hair dye products, is listed in the New Zealand Cosmetic Products Group Standard, Schedule 4 – Components cosmetic products must not contain (New Zealand EPA 2019).

United States of America

The trisodium salt, referred to as FD&C Red No. 2, no longer has provisional listing for use in food, drugs and cosmetics, according to US Code of Federal Regulations (CFR) Title 21, Part 81, Section 81.10 – Termination of provisional listings of colour additives (21 CFR 81.10).

Asia

The trisodium salt is listed in the Association of Southeast Asian Nations (ASEAN) Cosmetic Directive, Annex II – List of substances which must not form part of the composition of cosmetic products, where it must not be used as a substance in hair dye products, and in Annex IV, Part 1 – List of colouring agents allowed for use in cosmetic products (ASEAN 2021).

Other

The International Agency for Research on Cancer (IARC) monographs classify the trisodium salt, referred to as amaranth, as Group 3: Unclassifiable as to carcinogenicity to humans.

Health hazard information

Toxicokinetics

Two chemicals in the group, the free acid and the trisodium salt, are related by acid/base reactions and will be the same substance (pH dependent) in systemic circulations. It is assumed that the low solubility of the lake pigment relates to dissociation of the free dye from the pigment matrix.

For substances with sulfonic acid moieties each side of the azo bond, the aromatic amines produced following reduction of the azo bond would be sulfonated and; therefore, be water soluble, have low bioavailability through the skin and be readily excreted (NICNAS 2019).

The azo bonds of these chemicals in the group undergoes reductive cleavage releasing sulfonated aromatic amines based on in vivo studies showing one or more aromatic amines in the urine or faeces of certain mammalian species following oral exposure to the trisodium salt. There was little absorption of the intact chemical from the gastrointestinal tract of rats. The liver enzyme that reduces azo linkages plays only a small part in the metabolism, as was shown in experiments in which the trisodium salt was administered by intrasplenic infusion. Intestinal bacteria are probably responsible for most of the reduction of orally administered chemical. The major metabolite found in the plasma and urine and faeces is naphthionic acid (1-amino-4-naphthalenesulfonic acid, CAS No. 84-86-6). The other metabolite from reductive cleavage of the azo bind is expected to be 1-amino-2-hydroxy-3,6-naphthalenedisulfonic acid (CAS No. 135-51-3). The route of excretion is species dependent. In the rat and mouse, the principal route of excretion was the faeces, whereas in the guinea pig, urinary excretion accounted for up to 50% of the dose. No marked accumulation of naphthionic acid was found in any tissue of rats, mice or guinea pigs (EFSA 2010; Government of Canada 2016).

When the trisodium salt is administered intravenously, the chemical is rapidly excreted in the bile by an active process using the same pathways as other organic ions. Excretion occurs almost entirely as the unchanged chemical and is in part dependent on bile flow. The rapid uptake by the liver appears to be saturable and the rate limiting factor would be its removal from the biliary tree by bulk flow (EFSA 2010).

Although in some azo pigments reductive cleavage of the azo bond is not observed, monoazo pigments such as members of this group have greater enzymatic cleavage when compared to larger pigments and; therefore, may lead to cleavage products (NICNAS 2019).

Acute toxicity

Oral

Based on the available information for the trisodium salt, these chemicals are expected to have low acute oral toxicity.

In a non-guideline acute oral toxicity study, 4–5 male DDY mice were administered the trisodium salt in saline solution at 2000 mg/kg bw (without a control group). No mortality was observed up to a dose of 2000 mg/kg bw. The LD50 for acute oral toxicity in male mice was >2000 mg/kg bw (REACHa).

In a non-guideline acute oral toxicity study, pregnant CD-1 mice (4/dose) were administered the trisodium salt in water at doses of 0 or 2000 mg/kg bw. No mortality or clinical signs of toxicity were observed. The LD50 for acute oral toxicity in male mice was >2000 mg/kg bw (REACHa).

The LD50 for the trisodium salt was reported in the following non-guideline studies (REACHa):

- 10 mg/kg bw (highest dose tested) in CD-1 male mice
- 1000 mg/kg bw (highest dose tested) in Fischer 344 male rats
- 6000 mg/kg bw in rats.

Dermal

No data are available for chemicals in this group. Based on the low acute oral toxicity and expected limited bioavailability through the skin, these chemicals are expected to have low acute dermal toxicity.

Inhalation

No data are available to evaluate this endpoint.

Corrosion/Irritation

Skin irritation

Information is available for the trisodium salt which is not considered to be a skin irritant.

In a skin irritation study conducted similarly to OECD TG 404, rabbits (n = 9) (sex and strain unspecified) were treated with the trisodium salt at doses of 0.1% or 1% (control groups unspecified) with limited study details. No irritation or significant systemic toxicity was observed. The trisodium salt was considered not to be irritating to the skin (REACHa).

In an in vitro skin irritation study conducted in accordance with OECD TG 439 (in vitro reconstructed human epidermis (RhE) test method for skin irritation), the trisodium salt was applied with water to RhE, for an exposure period of 60 minutes, followed by an observation period of 42 hours. A mean tissue viability value of 100% was reported for the trisodium salt in this study, and it was determined to not be irritating to the skin. Interpretation of results obtained from OECD TG 439 studies do not allow for distinction between irritation and corrosion (REACHa).

Eye irritation

A guideline in vitro study for the trisodium salt shows that it may cause eye irritation. While this local effect cannot be read across for the other group members, sulfonic acids are strongly acidic, so it is anticipated that the free acid would also be irritating to the eyes. Sufficient data are not available to classify.

In a GLP-compliant in vitro eye corrosion study conducted according to OECD TG 492, the trisodium salt (neat; 50 mg) was topically applied to reconstructed human cornea like epithelium (RhCE) for 6 hours using the EpiOcular test method for the solids protocol. Tissue viability was measured following exposure. The mean tissue viability was determined to be 17.8%. Based on the decision criteria for this test (tissue viability >60% (for EpiOcular)), the trisodium salt is predicted to meet the criteria for serious eye damage or eye irritation (REACHa). This test does not differentiate between UN GHS Category 1 (serious eye damage) and UN GHS Category 2 (eye irritation). RhCE test methods show a high percentage of false positive results. Further information would be required for classification purposes.

Sensitisation

Skin sensitisation

Limited data are available for the trisodium salt. There is no evidence of skin sensitisation in a single non-guideline study. The in silico data indicates the free acid may have weak sensitisation potential.

In a skin sensitisation test in guinea pigs with limited study details, application of the trisodium salt (concentration unspecified) did not cause skin responses after induction or challenge. No other details were provided (REACHa).

In silico

The knowledge based expert system Deductive Estimation of Risk from Existing Knowledge (DEREK) Nexus version 6.0.1 was utilised to predict sensitisation potential of the free acid and its two expected cleavage products. Sensitisation for the azo dye was considered plausible but there was insufficient data to make an EC3 prediction. One metabolite was predicted to be a non-sensitiser and the other metabolite was predicted to be a weak sensitiser.

Based on the mechanistic profiling functionality of the OECD QSAR Toolbox, there were no structural alerts for protein binding for the free acid and the 2 expected cleavage products (OECD QSAR Toolbox version 4.2).

Repeat dose toxicity

Oral

These chemicals are not expected to cause serious systemic health effects following repeated oral exposure.

In a non-guideline combined chronic toxicity and carcinogenicity study conducted similarly to OECD TG 453, Wistar rats (90 control; 54/sex/treatment group) were administered the trisodium salt in feed to provide exposure doses of 0, 50, 250 or 1250 mg/kg bw/day for 111 weeks (males) or 112 weeks (females) after weaning. These rats were exposed to the same dose levels in utero, and their parents were similarly exposed to the same dose levels for 60 days before mating. There was a dose-related pink colouration of the fur and faeces due to the trisodium salt. Animals in the high dose group (1250 mg/kg bw/day) excreted more moist faeces than the controls. No significant difference in the mortality was observed between the control and treatment groups. At the start of the study, the weight of males in the high dose group was 4% less than the control group. From week 19, the females in the high dose group weighed marginally less than the control group, and this difference reached statistical significance on week 35 and the remainder of the study period. Animals of both sexes in the high dose group consumed more food and water than the controls, which often reached statistical significance. The reduced weight of the animals was due to a reduction in the absorption or utilisation of nutrients (Clode et al. 1987; EFSA 2010; REACHa).

The haematological changes observed included decreased packed cell volume in the male high dose group (6 months), and in males (12 months) and females (18 months) in all treatment groups. However, these changes were not considered adverse changes because they were not dose related or consistent between the sexes when all the treatment groups were included in the examination. Statistically significant higher haemoglobin levels were observed in the female high dose group.

The full caecum absolute and relative weights were higher in both sexes in the high dose group and in males in the mid dose group. These changes were accompanied by an increased weight of the caecum wall, which was statistically significant only in the high dose group. The caecum changes were considered to be adaptive changes, not adverse.

Urine concentration in males in the high dose group was higher than the control group, which was statistically significant after water deprivation and accompanied with reduced urine output. The levels of protein in the urine were significantly higher in males in the mid dose (250 mg/kg bw/day) group and in females in the high dose group than the control group.

Non-neoplastic lesions were observed in males in all of the treatment groups with a dose related trends including transitional cell hyperplasia of the bladder and inflammatory cell infiltrate of the seminal vesicles. Testicular interstitial cell hyperplasia was observed in the low dose (50 mg/kg bw/day) and high dose groups.

The incidences of renal calcification and renal pelvic epithelial hyperplasia were significantly increased in females in all treatment groups compared to the control group. The incidences of lung oedema and haemorrhage, lymph node haemorrhage, degenerative changes in the brain and nerves, degenerative and inflammatory changes in the heart, aortic calcification, atrial thrombi, and inflammatory changes in the thymus were significantly increased in

females in the high dose group. Changes in the absorption or utilisation of minerals including calcium may have led to the renal calcification. In a re-evaluation of renal calcification and hyperplasia, a no observed adverse effect level (NOAEL) of 50 mg/kg bw/day was identified by the authors (Clode et al. 1987; EFSA 2010; REACHa).

To investigate the effects of increased calcium intake on renal calcification, a repeated oral dose toxicity study was undertaken with groups of 25 male and female Wistar rats. They were fed regular diets designed to provide intakes of 0, 20, 40, 80 or 1250 mg/kg bw/day of the trisodium salt for either 28 or 90 days. A positive control group of 25 rats of each sex was fed lactose at a dietary concentration of 25% for 4 days and then at 50% for a further 24 days. A significantly increased incidence of renal pelvic calcification and pelvic hyperplasia were observed in males in the positive control group at 28 days. Males in the high dose group (1250 mg/kg bw/day) gained slightly less weight than controls, but their food intake was unaffected. Relative kidney weights and the renal concentrations of calcium, magnesium and phosphorus were not affected by treatment of the trisodium salt at any dose level and for either period of time (28 or 90 days). A significantly increased number of high dose male animals with renal pelvic hyperplasia and calcification were observed at 90 days. This suggests that the renal effects at 90 days may be due to the trisodium salt adversely affecting the kidneys of ageing animals. The NOAEL for males was 80 mg/kg bw/day, and for females, was 1250 mg/kg bw/day (Clode et al. 1987; EFSA 2010).

In a non-guideline repeated oral dose toxicity study, Sprague Dawley (SD) female rats (10/dose) were orally administered the trisodium salt in water at exposure doses of 0, 4.7 or 47 mg/kg bw/day for 2 months. A significantly increased serum alkaline phosphatase levels was observed at a dose of 47 mg/kg bw/day compared to the control group; however, it is unclear if this is an adverse or adaptive as there were no other tests conducted to investigate the underlying cause. No significant changes were observed in serum alanine aminotransferase and aspartate aminotransferase levels in any of the treatment groups. No significant changes were observed in serum urea, creatinine and in several haematological parameters (erythrocyte count, haemoglobin concentration, packed cell volume (%), mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and total leucocyte count). The NOAEL for female SD rats was 47 mg/kg bw/day which was the highest dose tested (Hashem et al. 2011).

In a non-guideline combined repeated dose toxicity with reproduction and developmental toxicity screening study, Crj:CD-1 mice (10/sex/dose) was administered the trisodium salt in feed at doses of 0, 50, 150 or 450 mg/kg bw/day (equivalent to dietary levels of 0, 0.03%, 0.09% and 0.27%) for a total of 14 weeks. A number of spontaneous motor activity parameters were reduced in the parental males in the high dose group (450 mg/kg bw/day), and in the mid (150 mg/kg bw/day) and high dose parental females compared to the control group. Increased spontaneous motor activity parameters observed in the mid and high dose group females included number of movements, average distance and average speed. The NOAEL for both parental sexes was 50 mg/kg bw/day (equivalent to a dietary level of 0.03%) in Crj:CD-1 mice (EFSA 2010; REACHa; Tanaka 1992).

Dermal

These chemicals are not expected to cause serious systemic health effects following repeated dermal exposure.

In a non-guideline repeated dermal toxicity study, the trisodium salt was topically applied at a concentration of 0 or 1% on to the clipped skin (6 cm^2) of Swiss Webster mice (100/sex for control group; 50/sex for treatment group) once a week for 18–19.5 months. No adverse reactions or pathological changes were observed after 19.5 months of dermal exposure to

the trisodium salt. The no observed adverse effect concentration (NOAEC) was 1% for both sexes of Swiss Webster mice (REACHa).

Genotoxicity

These chemicals are not expected to have genotoxic potential.

Sulfonation of the ring of azo dyes is considered to prevent the activation of the resulting aromatic amines to genotoxic and carcinogenic products. The metabolites of the trisodium salt, naphthionic acid and 2-naphthol-3,6-disulfonic acid, sodium salt (R-amino salt), were considered to be non-mutagenic (EFSA 2010). This is supported by in silico data.

In vitro

Negative results were reported in the following in vitro genotoxicity studies (REACHa):

- Six bacterial reverse mutation tests conducted similarly to OECD TG 471 in *Salmonella typhimurium* strains (including TA92, TA94, TA97a, TA98, TA100, TA1535, TA1537) up to 10000 µg/plate with or without metabolic activation.
- A bacterial reverse mutation test conducted similarly to OECD TG 471 in *S.typhimurium* strain YG1024 with and without metabolic activation, and TA100 with and without metabolic activation.

Positive results were reported in the following in vitro genotoxicity studies (REACHa):

- A bacterial reverse mutation test conducted similarly to OECD TG 471 in *S. typhimurium* strain TA98 with metabolic activation.
- An in vitro mammalian chromosome aberration test conducted in Chinese hamster lung fibroblasts (V79) up to 1.0 mg/mL without metabolic activation.
- An in vitro mammalian chromosome aberration test conducted in V79 cells up to 250 µg/mL without metabolic activation (REACHa).

In vivo

In a mammalian bone marrow chromosome aberration test conducted similarly to OECD TG 475, Swiss albino male mice (4/dose) were administered the trisodium salt intraperitoneally at doses of 0, 50, 100 or 200 mg/kg bw. The incidence of chromosome aberrations in bone marrow did not increase in any of the treated groups, indicating a lack of clastogenicity (REACHa).

In a non-guideline gut micronucleus study, Swiss male mice were administered the trisodium salt by oral gavage at doses of 0, 20, 200 or 1000 mg/kg bw twice 24 hours apart. The incidence of micronuclei in the gut did not increase in any of the treated groups, indicating a lack of clastogenicity (REACHa).

In a non-guideline rat hepatocyte primary culture DNA repair assay, SD rats (n = 6–8) were administered the trisodium salt at 0 or 200 mg/kg bw by oral gavage and their hepatocytes were obtained for culture. There were no signs of DNA damage in liver cells at the dose tested (REACHa).

In silico

The (Q)SAR modelling for genotoxicity using the OECD QSAR Toolbox version 4.2 there were structural alerts for in vivo mutagenicity (micronucleus) for the free acid and one expected cleavage product.

The knowledge based expert system DEREK Nexus version 6.0.1 was utilised to predict genotoxicity potential of the free acid and its 2 expected cleavage. There were no alerts for mutagenicity in vitro (no misclassified or unclassified features) and these chemicals were; therefore, considered negative for mutagenicity.

The QSAR predictions using OASIS TIMES indicate that the free acid and its S9 metabolites were negative for mutagenicity (Ames) in the in vivo micronucleus test. The predictions were within the applicability domain of the genotoxicity models.

Carcinogenicity

Available information indicates that chemicals in this group are not carcinogenic.

In a non-guideline combined chronic toxicity and carcinogenicity study, Wistar rats (90 control; 54/sex/treatment group) were administered the trisodium salt in feed to provide exposure doses of 0, 50, 250 or 1250 mg/kg bw/day for 111 weeks (males) or 112 weeks (females) (see **Repeated Dose Toxicity – Oral** section). The rats had also been exposed to the same dose levels in utero, and their parents were exposed for 60 days before mating. In males, no primary tumour site showed a significantly increased incidence in treated rats compared with the controls. The incidence of uterine polyps and vaginal fibromas was significantly increased in the high dose group compared to the control group, which the authors considered to be strain dependent and; therefore, unlikely to be treatment related. Overall, the incidence and organ distribution of tumours found in the study were typical for the strain of rat used in the study and did not show a treatment related effect (Clode et al. 1987; EFSA 2010; REACHa).

In a non-guideline repeated dose dermal toxicity study, the trisodium salt was topically applied at a concentration of 0 or 1% on to the clipped skin of Swiss Webster mice (see **Repeated Dose Toxicity – Dermal** section) once a week for 18–19.5 months. No increase in the incidence of neoplasia following repeated exposure to the trisodium salt was observed compared to the control group. The NOAEL was 1% for both sexes of Swiss Webster mice (REACHa).

The working group of the IARC reviewed a number of non-guideline carcinogenicity studies in rats, mice and dogs for the trisodium salt (IARC 1975). Animals were exposed to the trisodium salt in the diet or sub-cutaneously. In the majority of studies no tumours were observed. A number of limitations in these studies were noted including numbers of animals, duration of study or insufficient reporting. In one study an increase in tumours of the peritoneum and intestine was reported when rats were fed diets containing up to 1.6% of the trisodium salt (25–35% impurities), for 25 months. No tumours were seen in controls. In a similar study, rats given diets containing 2% “chemically pure” amaranth for their lifespan (up to 33 months) developed a variety of malignant tumours while no tumours were recorded in controls. Both IARC and EFSA questioned the validity of these studies as:

- tumours were not related to a specific organ and were representative of the background neoplasia seen in aged rats
- the absence of tumours in control animals in both studies was very unusual

- in the first study there was a large percentage of impurities (EFSA 2010; IARC 1975).

EFSA considered that the available studies do not indicate that amaranth has carcinogenic potential (EFSA 2010).

Reproductive and development toxicity

These chemicals are not expected to cause specific adverse effects on fertility and development.

In a prenatal development study, pregnant New Zealand White (NZW) rabbits (17/dose) were orally administered the trisodium salt as a gelatin capsule at doses of 0, 1.5, 5.0 or 15 mg/kg bw/day from gestation day 6–15. On gestation day 29, the animals were sacrificed and the foetuses were removed by caesarean section. No signs of maternal toxicity were observed in any of the animals. The maternal body weight was not adversely affected by the trisodium salt treatment. The number of viable offspring from all treatment groups appeared to be slightly lower than the control group. The body weights and 24 hour survival of the offspring were not affected by the treatment. No significant increase in the incidence of gross anomalies in the foetuses or effects on skeletal foetal development were observed. The NOAEL for both maternal and foetal effects was 15 mg/kg bw/day (REACHa).

In a prenatal development study with limited study details, pregnant SD rats (10/dose) were orally administered the trisodium salt by gavage at doses of 0 or 47 mg/kg bw/day from gestation days 6–15. No data on maternal effects were reported. Abnormalities in the foetuses exposed to the trisodium salt at 47 mg/kg bw/day in utero included growth retardation (in 27.8% of foetuses), hypoplasia of the heart and lungs (in 8.3% of foetuses), incomplete ossification of the skull bones, aplasia of metacarpal and metatarsal bones and of the caudal vertebrae (in 25% of foetuses). None of these abnormalities were observed in the control group. The LOAEL for the foetal effects was 47 mg/kg bw/day (Hashem et al. 2011; REACHa).

EFSA (2010) assessed the available teratogenicity and multigenerational reproduction studies. Due to methodological insufficiencies, many of them were not conclusive for the determination of any reliable NOAEL in the rat, mouse, hamster and rabbit. Several studies were negative in terms of reproduction toxicity in the rat, or developmental toxicity in the mouse, rabbit and dog. Consequently, the highest dose tested in these studies was considered to be the NOAEL (EFSA 2010).

There have been frequent observations of increased resorptions indicating embryotoxicity of amaranth, but repetition of the experiments with improved experimental designs have usually failed to confirm this. Taking all the reproduction and developmental studies into account, NOAELs for amaranth can be identified in the following species tested: mouse 100 mg/kg bw/day (highest dose tested), rat 15 mg/kg bw/day, rabbit 15 mg/kg bw/day (highest dose tested), cat 50 mg/kg bw/day and dog 75 mg/kg bw/day (approximately) (EFSA 2010).

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